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HYPOTONICITY INDUCED HUMAN RED BLOOD CELL (HRBC) MEMBRANE STABILIZATION POTENTIAL OF CRUDE ETHANOLIC EXTRACT OF CINNAMOMUM VERUM

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ABSTRACT --*Cinnamomum verum may be a common species. It cures respiratory disease, digestive discomfort and gynaecological syndrome. Inflammation may be a biological response of the system to harmful stimuli and it's characterised by redness, swelling, pain and loss of tissue function. Now -a -days NSAIDS is employed widely to stop inflammation. But it has more side effects. Due to this side effect, it's necessary to seek out a natural anti-inflammatory agent. to work out the anti-inflammatory activity in crude Ethanolic extract of Cinnamomum verum . Cinnamon verum was purchased from herbal cure centre . Ethanolic extract of Cinnamon verum was prepared and its anti-inflammatory activity was evaluated by hypotonicity induced HRBC membrane stabilization.: As observed from the study, we can conclude that cinnamomum verum has anti-inflammatory activity. Cinnamomum verum exhibits anti-inflammatory activity by stabilizing the lysosomal membrane.*

KEY WORDS*-- Cinnamomum verum , Non-steroidal anti-inflammatory drug , HRBC membrane stabilization ,Diclofenac sodium , anti-inflammatory activity .*

I. INTRODUCTION

Cinnamon verum (synonym. Cinnamon zeylanicum) is a small evergreen tree .Cinnamon is a common species (1) and it’s official name in Tamilnadu is Ilavangam . It’s used daily by people everywhere. The plant Cinnamomum is from the Lauraceae family. The dominant constituent of cinnamomum is crucial oil, cinnamaldehyde ,cinnamic acid and cinnamate . Cinnamomum is majorly utilized in perfume and scent stores (2,) .Cinnamomum is popularly used as tooth powder and to treat dental problems and fight against bad breath (3).Cinnamomum plays an important role against neurological disorders like Alzheimer and Parkinson’s Syndrome .Coumarin are dependent phytochemicals with active anti-coagulant and hepato -toxic property(4) . Cinnamomum is high in antioxidant activity (5) and has antimicrobial property (6) . So, it's used as preservatives in food. Cinnamon boosts blood circulation within the uterus and proposes tissue regeneration (7). Its a coagulant and prevents bleeding (8). It has several flavonoid rich compounds like gossypin , gnaphalium , hesperidin , bifolin ,

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hypolaetin , oroxidin and quercetin . These compounds have anti-inflammatory activity . Recent study reported that 2' hydroxy cinnamaldehyde exihibited an inhibitory effect on the activation of the nuclear factor Kappa -light - chain enhancer of activated B - cells , indicating that this substance can potentially be used as an anti-inflammatory agent(9) . Inflammation may be a physiologic series of responses generated by the host in response to infection or other insults and characterised by redness ,swelling,heat ,pain and loss of function (10) . Lysosomal enzymes released during inflammation produce a spread of disorders which results in the tissue injury by damaging the macromolecules and lipid peroxidation (11) .Anti oxidant decreases under some inflammatory condition which may be built up by foreign source (12) Inflammation are the results of vasodilation and increased vascular permeability , resulting in exudation of fluid and protein recruitment of leukocytes to the location of injury (13) .Stabilization of lysosomal membrane is vital in limiting the inflammatory response by inhibiting the discharge of lysosomal constituents of activated neutrophil like bactericidal enzymes and proteases which cause further tissue inflammation and damage upon extracellular release or by stabilizing the lysosomal membrane (14). Now -a -days Non steroidal anti-inflammatory drug (NSAIDS) is employed widely to stop inflammation.The use of NSAIDS , has not been therapeutically successful in decreasing thge conditions of inflammation (15). Usage of NSAIDS commonly utilized in acute and chronic pain in dentistry (16) . But it has more side effects like increased risk of attack , rheumatic disease , causes stroke and should cause ulcers or bleeding within the stomach or intestine (17) . Due to this side effect, it's necessary to seek out a natural anti-inflammatory agent . Various plant extracts and their isolated compounds are proved nearly as good as synthetic anti-inflammatory agents (18) .Our present work aims at evaluating the in vitro anti-inflammatory activity of crude Ethanolic extract of Cinnamomum verum by Stabilisation of human red blood corpuscle membrane (HRBC) by hypotonicity induced membrane lysis .

II. MATERIALS AND METHOD

Cinnamomum verum spice is collected from local stores . It had been weighed for 25 grams and made with crushed powder with the assistance of mortar and pestle . The 25 grams cinnamomum bark was placed into a flask and the Ethanol solvent was added to the flask . The filtrate was concentrated to dryness under reduced pressure at 40^0C employing a rotary evaporator.

In vitro hypotonicity induced HRBC membrane stabilization potential of C.verum

Preparation of Human Red Blood Cells (HRBC) Suspension

Fresh whole human blood was collected and mixed with equal volume of sterilized Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.05% citric acid and 0.42 % sodium chloride in water). The blood was centrifuged at 3000 rpm for 10 min and packed cells were washed three times with isosaline (0.85%, pH 7.2). The volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline.

Hypotonicity Induced Human Red Blood Cell (HRBC) Membrane Stabilization Method

The principle involved here is stabilization of human red blood cell membrane by hypo tonicity induced membrane lysis. The assay mixture contains 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline [0.36 %], 0.5 ml HRBC suspension [10 % v/v] with 0.5 ml of plant extracts and standard drug diclofenac sodium of various

concentrations (50, 100, 250, 500, 1000, 2000 µg/ml) and control (distilled water instead of hypo saline to produce 100 % hemolysis) were incubated at 37degree Celsius for 30 min and centrifuged respectively. The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm. The percentage of hemolysis of HRBC membrane was calculated as follows: % Hemolysis = (Optical density of Test sample / Optical density of Control) X 100.

The percentage of HRBC membrane stabilisation can be calculated as follows:

% Protection = 100 – [(Optical density of Test sample / Optical density of Control) X 100].

III. STATISTICAL ANALYSIS

The data were be subjected to statistical analysis using one-way analysis of variance (ANOVA) and Duncan's multiple range test to assess the significance of individual variations between the groups. In Duncan's test, significance was considered at the level of $p<0.05$.

IV. RESULTS

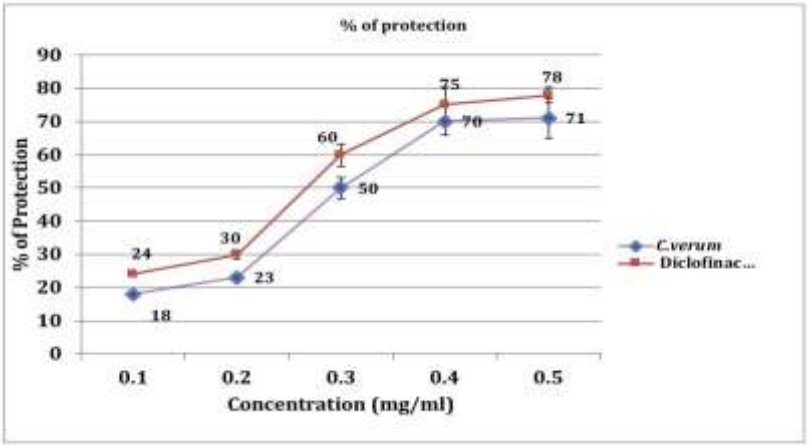

Figure 1: Effect of Cinnamomum verum on HRBC membrane stabilization

Each line represents Mean SEM of 3 independent observations. Significance at p.0.05.

In this graph, we can see the action of Cinnamomum verum and Diclofenac sodium which is a standard drug on HRBC membrane stabilization or protection . Here, the standard drug shows more protection than natural drugs

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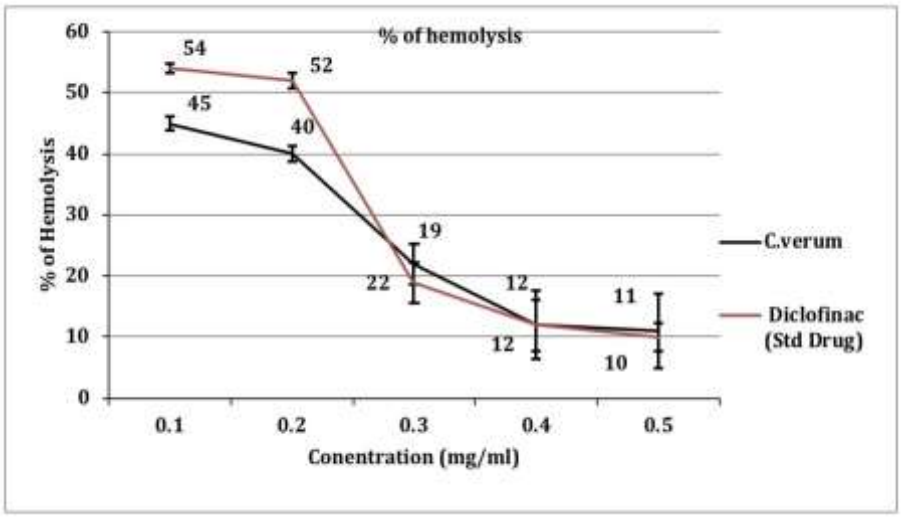

Figure 2: Effect of Cinnamomum verum on hemolysis activity

Each line represents Mean SEM of 3 independent observations. Significance at p.0.05 .

In this graph , we can see the action of Cinnamomum verum and Diclofenac sodium on hemolysis which is a death of Red Blood cells . Here ,also the standard drug inhibits more number of RBC lysis than Cinnamomum verum .

V. DISCUSSION

Inflammation may be a physiologic series of responses generated by the host in response to infection or by other foreogn particle.The lysosomal enzymes are released during inflammation which produce sort of disorders . The non steroidal drugs act by inhibiting or stabilising the lysosomal membrane. Since HRBC is the same as lysosomal membrane components like lipids,proteins ,Nucleic acids and carbohydrates. The prevention of hypotonicity induced HRBC membrane Stabilisation is employed as an anti-inflammatory agent . Anti inflammatory activity of Cinnamomum verum containing gossypin , gnaphalium , hesperidin , bifolin , hypolaetin , oroxidin and quercetin shows significant effect at higher concentration .Anti inflammatory drugs exhibit membrane stabilisation effect by inhibiting hypotonicity induced lysis of erythrocyte membrane , the erythrocyte membrane is analogous to lysosomal membrane and its stabilisation implies that drug may also stabilize lysosomal membrane. Moreover , Stabilisation of the lysosomal membrane is vital in limiting the inflammatory response by preventing the discharge of lysosomal constituents of activated neutrophil like bacterial enzymes and protease ,which cause tissue inflammation . In present study ,the effectiveness of Cinnamon verum extract on HRBC membrane stabilisation was analsed. 0.1 mg/ml of concentration shows 18% protection ; 23% protection at concentration of 0.2 mg/ml ; 50% protection at concentration of 0.3 mg/ml ; 70% protection at concentration of 0.4 mg /ml and 71% protection at concentration of 0.5 mg/ml , respectively .(Fig 1) while the effect of Diclofenac sodium on HRBC membrane Stabilisation for 0.1 mg / ml of concentration shows 24% protection ; 30% protection at concentration of 0.2 mg/ml ; 60% protection at concentration of 0.3. mg /ml ; 75 % protection at concentration of 0.4 mg /ml and 78 % protection at concentration of 0.5 mg/ml , respectively. When we compare this natural drug protection with Diclofenac sodium which we used as a typical drug , it shows more protection than natural

drug .The effect of Cinnamon verum on inhibition of hemolysis was studied and found to be 45% hemolysis at concentration of 0.1mg/ml ; 40% hemolysis at concentration of 0.2 mg/ml ;19% hemolysis at concentration of 0.3 mg /ml ; 12% hemolysis at concentration of 0.4 mg/ml and 11% hemolysis at concentration of 0.5 mg/ml, respectively . While the effect of ordinary drug on hemolysis activity shows 54 % hemolysis at concentration of 0.1mg/ml ; 52%hemolysis at concentration of 0.2 mg/ml ;22% hemolysis at concentration of 0.3 mg /ml ; 12% hemolysis at concentration of 0.4 mg/ml and 10 % hemolysis at concentration of 0.5 mg/ ml, respectively . As the concentration increases , protection or Stabilisation also increases as shown in Fig .1 and hemolysis reduces at higher concentration as shown in Fig .2 Previous studies on anti inflammatory activity has also proved the effectiveness of the various natural herbal extracts against inflammation. Therefore ,this natural drug acts as a better anti-inflammatory agent .But it has no side effects which is a daily required product and it has numerous medicinal properties.

VI. CONCLUSION

From the study , it's evident that the ethanolic extract of Cinnamomum verum can stabilize the lysosomal membrane . This extract protects the human erythrocyte membrane against lysis . Thus, the extract act as an anti-inflammatory agent . Though it is less effective than standard drug it has no side effect .Therefore , the ethanolic extract of Cinnamomum verum being a spice can be used as a food additive . The extract can be further studied for its anti-inflammatory potential invivo .

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